AD_____

Award Number: DAMD17-97-C-7052

TITLE: Analytical and Characterization Studies of Organic Chemicals, Drugs and Drug Formulation

PRINCIPAL INVESTIGATOR: Peter Lim, Ph.D.

CONTRACTING ORGANIZATION: SRI International
Menlo Park, CA 94025

REPORT DATE: February 2004

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2004	Final (1 Jul 1997 - 6 Jan 2004)

4. TITLE AND SUBTITLE

Analytical and Characterization Studies of Organic Chemicals, Drugs and Drug Formulation

5. FUNDING NUMBERS

DAMD17-97-C-7052

6. AUTHOR(S)

Peter Lim, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

SRI International
Menlo Park, CA 94025

E-Mail: peter.lim@sri.com

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The overall purpose of this contract was to perform chemical/physical analyses on bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, etc. Specific objectives were to design, develop, validate, and apply methods to determine chemical and physical characteristics on bulk drugs and drug products.

For the entire contract period, 1 July 1997 to 6 January 2004, 117 samples of bulk drugs and dosage formulations were analyzed for identity and purity or potency; 31 samples were studied for bulk stability, solubility and solution stability. Chiral chromatographic separation methods were developed and validated for five bulk drugs. Extensive dissolution studies in four media were performed on five batches of tablets. A special project was development of methods to isolate anti-malarial agents from rabbit plasma and applying the methods to determine these drugs in 128 samples of rabbit plasma. A second and much more important special project was development of a highly sensitive and specific method to determine squalene in 17 lots of Anthrax Vaccine Adsorbed. As a result of this work, several awards were presented to members of the project team and to SRI International. A third special project was development and production of a formulated drug product for artesunic acid. Two batches of this drug product were produced and sent to the Army for toxicological evaluations. A poster presentation was made at the 2000 Bioscience Review, and one publication appeared in press.

14. SUBJECT TERMS

Antiparasitic Drugs, Chemical Defense Agents, Chemical Analyses, Stability Studies

15. NUMBER OF PAGES

10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

This final report for Contract DAMD17-97-C-7052 covers the period from 1 July 1997 to 06 February 2004. The report consists of a listing of the compounds/samples analyzed and a summary of the number of the types of studies performed. The report also includes a listing of personnel receiving pay from this effort and a bibliography of all publications and meeting abstracts that resulted from this contract.

This contract was concerned with the analytical, characterization, and stability studies of chemicals, drugs and drug formulations. The work was monitored by Mr.William Y. Ellis, the Contracting Officer Representative (COR), Chief, Department of Chemical Information, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR).

The overall objective of this project is the operation of an analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense studies, etc. Specific objectives are to design, develop, validate, and execute methods to determine the following characteristics of candidate bulk pharmaceutical substances and formulated drugs:

- Identity, purity, and strength
- Stability
- Other physical and chemical characteristics, including weight variation, content uniformity, and other such compendial requirements
- Qualitative and quantitative identity of impurities
- Special projects not covered by the above headings

FINAL REPORT

Sample Analyses

During the contract period, 1 July 97 – 6 Jan 04, analyses of the following samples were completed and the reports sent to the COR.

1. WR1544, chloroquine diphosphate, Reports Nos. 968, 970, 971, and 972.

2. WR2975, primaquine phosphate tablets, Report No. 1051.

3. WR6026, 6-methoxy-8-(6-diethylaminohexylamino)lepidine dihydrochloride, Report Nos. 941, 942, and 943.

4. WR7930, α-2-piperidinyl-6,8-dichloro-2-phenyl-4-quinolinemethanol monohydrochloride, Report No. 1017.

5. WR35928, paromomycin sulfate, Report No. 1040.

6. WR73633, gentamicin sulfate, Report No. 1053.

7. WR178460, desbutylhalofantrine hydrochloride, Report Nos. 991, 992, 1027, 1025, 1026, 1031, 1032, 1034, 1035, and 1036.

8. WR229870, antimony sodium gluconate (Pentostam), four lots of this drug product from Burroughs Wellcome have been assayed and preliminary reports have been forwarded. Pentostam is also undergoing a long-term study.

9. WR238605, N^4-(2,6-dimethoxy-4-methyl-5-(3-(trifluoromethyl)phenoxy-8-quinolinyl)-1,4-pentanediamine succinate, Report Nos. 944, 945, and 969.

10. WR242511, 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL tartrate, Report Nos. 982, 984, 985, 987, 980, 981, 1016, and 1018.

11. WR243246, 7-chloro-3*-(2",4"-dichlorophenyl)-1,2,3,4-tetrahydro-1,9-(10H)acridindione, Report No. 958.

12. WR243251, 7-chloro-3-(2",4"-dichlorophenyl)-1-[[3'-(dimethylamino)propyl]imino]-1,2,3,4-tetrahydro-9-(10H)acridinone, Report Nos. 958 and 1016.

13. WR249309, artemisinin (qinghaosu) , Report No. 978.

14. WR249655, 3-(4-carbamoyl-1-pyridino)-1-(2-hydroxyiminomethyl-1-pyrinino)-2-oxapropane dimethansulfonate (HI-6 diMesylate), two letter reports.

15. WR250547, (R)-7-chloro-3*-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-[[3-(dimethylamino)propyl]imino]-9-acrindinol, Report No. 990.

16. WR250548, (S)-7-chloro-3*-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-[[3-(dimethylamino)propyl]imino]-9-acridinol, Report No. 989.

17. WR250710, 3-[[(dimethylamino)carbonyl]oxy]-1-methylpyridinium bromide, (pyridostigmine bromide), Report Nos. 993 and 1050.

18. WR253648, 1-(4-aminocarbonylpyridino)-3-(2-hydroxyiminomethylpyridino)propane dichloride, a letter report.

19. WR255131, β-arteether, Report No. 1057.

20. WR255608, methyl artelinate, Report No. 967.

21. WR255663, 4-(10'-dihydroartemisininoxymethyl)benzoic acid (artelinic acid) Report Nos. 959, 973, 974, 995, 996, 997, 1023, 703 revised, and 1047.

22. WR256042, 1-[2'-[(hydroxyimino)methyl]-3'-methyl-1'-imidazolyl]-3-(4"carbamoyl-1"-pyrindinyl)propane dichloride monohydrate, two letter reports.

23. WR.256283, dihydroqinghaosu hemisuccinate (artesunic acid), Report Nos. 1037, 1072, and 1073.

24. WR268384, 2,5,5-trimethyl-2-[2'-(4'-carboxymethyl-1"-R-methyl-3"-trimethylsilylmethylenechclohex-2"-yl)ethyl]1,3-dioxane, Report No. 1071.

25. WR279396, paromomycin sulfate/gentamicin sulfate cream , Report Nos. 938, 939, 940, 1019, 1021, 1056, 1058, and 1059.

26. WR282644, α-artelinic acid, Report Nos. 996 and 997.

Special Projects

Special projects requiring extensive methods development and applying the developed methods to assay large numbers of samples include:

A. Determinations of Anti-malarials in Rabbit Plasma

1. Development of a method to extract WR142490, mefloquine (hydrochloride), from rabbit plasma and a chiral chromatographic procedure to separate the extracted mefloquine enantiomers, Report No. 960.

2. Development of a method to extract WR2976, quinine sulfate, from rabbit plasma and a chromatographic procedure to separate the extract components, Report No. 961.

3. Employing a method to extract WR171669, (±) halofantrine, and WR178460, (±)desbutylhallfantrine, from rabbit plasma provided to us by Professor Emil Lin at UCSF, we developed a chromatographic method to separate and quantify the titled analytes in the extract, Report No. 962. Using the described procedures, the halofantrine/desbutylhalofantrine contents in 128 samples of rabbit plasma were determined, Report No. 979.

B. Determination of Squalene in Samples of Anthrax Vaccine

A highly specific and sensitive method for determining squalene in anthrax vaccine preparations was developed, validated, and applied to 61 samples of 17 lots of Anthrax Vaccine Adsorbed supplied by the Army. No squalene has been detected in any sample in any lot within the method's lower limit of detection, which is 70 ng squalene/0.5 mL vaccine dose or 140 parts per billion (ppb). These studies are reported in Report Nos. 983, 983, 983R, 994, 999, 999R, 1001, 1001R, 1002, 1002R, 1003, 1003R, 1004, 1004R, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1033, and 1049.

The same method was applied to samples of a commercial flu vaccine, FLUAD®, which is labeled to contained squalene as adjuvant; the amount of squalene found approximated the labeled quantity.

Stability and Solubility Studies

1. WR171669, halofantrine hydrochloride, completed a 10-year shelf-life stability study, Report No. 986.

2. WR178460, desbutylhalofantrine hydrochloride, for shelf-life stability study, Report Nos. 966, 975, 1027, and 1032; for 35°C stability study, Report Nos. 946, 976, 991, 1025, and 1034; and for 50°C stability study, Report Nos. 947, 977, 992, 1026, and 1035.

3. WR242511, 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL tartrate, shelf-life stability study, Report Nos. 981 and 1018.

4. WR255663, β-artelinic acid, shelf-life stability study, Report Nos. 965, 1020, 1052, and 1061; thermal and shock stabilities, Report Nos. 949 and 949R; stabilities of 1% and 4% artelinic acid in solutions of lysine in saline at room temperature, 35°C, and 50°C, Report Nos. 1046, 1049, and 1055; A sample of sodium artelinate was prepared and its room-temperature solubility and solution stability were determined, Report No. 1015.

5. WR256283, dihydroqinghaosu hemisuccinate (artesunic acid), room-temperature solubility in 5% sodium bicarbonate/5% glucose and solution stability were studied in detail for a commercial drug product and samples of bulk drug, Report Nos. 1036 and 1068; extensive solubility and stability/kinetic studies of bulk artesunic acid in phosphate solutions, differing

in pH and phosphate concentrations, were carried out, and results from these studies eventually led to a formulation of artesunic acid, Report Nos. 1038, 1041, and 1054.

6. WR279396, paromomycin/gentamicin in a cream formulation, determination of physical stability of the cream product and of the chemical stabilities of the aminoglycoside antibiotics after storage at 4±°2°C, Report No. 998.

Chiral Chromatographic Separations and Method Validations

1. WR178460, desbutylhalofantrine hydrochloride, chiral separation of (±) racemic desbutylhalofantrine into its (-) and (+) enantiomer(s), Report No. 918.

2. WR280510, (R)-8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL tartrate, validation of chiral separation method, Report No. 950.

3. WR242511, 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL tartrate, re-validation of assay method, Report No. 980.

4. WR280511, (S)-8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL tartrate, validation of chiral separation method, Report No. 951.

5. WR280691, (-)desbutylhalofantrine hydrochloride, validation of chiral separation method, Report No. 936.

6. WR280823, (+)desbutylhalofantrine hydrochloride, validation of chiral separation method, Report No. 937

Extensive Comparative Dissolution Studies

Dissolution studies in four different media for five batches of pyridostigmine bromide tablets were completed, and the results from all five batches were compared in detail, Report Nos. 1048, 1062, 1064, 1065, 1066, and 1067.

Development and Production of a Formulated Product of WR256238, Artesunic Acid

In cooperation with members of the SRI's Formulations Development Department, we carried out extensive formulation development on artesunic acid. The effort resulted in a product for which a unit dosage consists of a vial containing 500 mg of dry artesunic acid and a vial containing 10.0 mL 300 mM, pH 8.1 sodium phosphate solution. When the two vial contents are admixed until a solution results, and the solution sterile filtered, the filtrate contains 50 mg artesunate (sodium)/mL solution, which is the labeled concentration. This product meets the Army's requirement for potency, pH, particulate count (including air bubbles), osmolality, stability, and most importantly rate of dissolution.

Two batches of this product were manufactured and shipped to the Army, along with the respective Batch Record Reports, which include the assay results. The first batch, Batch No.14290-15, consists of 175 vials of dry-filled artesunic acid and 350 vials of 300-mM phosphate solution; the second batch, Batch No. 14290-16, consists of 482 vials of dry-filled artesunic acid and 966 vials of 300mM phosphate solution.

Currently in progress is a development of a 10 mg artesunate/mL solution formulation. Because of critical requirements imposed on this product, which also will be a two-vial unit dosage, the contents in both vials need to be sterile from the time of production to the time of administration-- as sterile filtration just prior to administration is an unacceptable operation. Owing to sterility and stability demands placed on this product, particularly on the artesunic acid portion, its development cannot be a mere modification of the production protocol to the above product. Although progress has been slow, it is beginning to show signs of promise.

Portable Document Format (PDF) Reports

Two hundred and five (205) completed technical reports in electronic portable document format (PDF) were uploaded to the ETIMAGE server at WRAIR.

SOP Updates

Updating of 25 SOP's were continuously performed as needed throughout the contract period.

Presentations and Publications

A poster entitled "Development and Application of an Analytical Methodology to Verify the Absence of Squalene in Anthrax Vaccine Adsorbed Formulations" was presented at the U.S. Army Medical Defense Bioscience 2000 Review.

A publication entitled "Development and Application of An Analytical Method for the Determination of Squalene in Formulation of Anthrax Vaccine Adsorbed" has been published in Journal of Pharmaceutical and Biomedical Analysis, 29/1-2, pp. 183-193 (2002).

Awards

For contributions to the resolution of the vaccine/squalene problem, project team members Ronald Spanggord, Benjamin Wu, and Peter Lim are granted honorary memberships in the United States Army Medical Department Regiment by order of the Surgeon General, Lieutenant General Ronald R. Blanck.

Moreover, SRI International received a certificate from the United States Army Medical Department Regiment recognizing SRI as a "Friend to the Regiment" for outstanding support provided to the Army Medical Department.

Additionally, Peter Lim is presented the Commander's Award for public service.

PERSONNEL

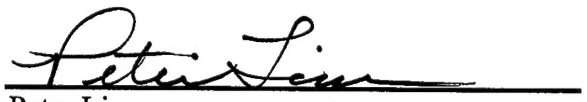
A listing of personnel who received major contract support during the report period is as follows:

Peter Lim, P.I.
Ronald Spanggord, Assistant P.I.
Lori Olson, Assistant P.I.
Patrick Macauley, Chemist
John Pick, Chemist
Tina Nguyen, Chemist
Benjamin Wu, Chemist
Mario Magon, Chemist
Meg Sun, Chemist
Laura Rasay, Chemist
Christine Salvatore, Formulation Chemist II

SUMMARY/CONCLUSION

During the entire contract period, 117 samples of bulk drugs and dosage formulations were analyzed for identity and purity or potency; 31 samples were studied for bulk stability, solubility and solution stability, and chiral chromatographic separation methods were developed and validated for five bulk drugs. Extensive dissolution studies in four media were performed on five batches of tablets. A special project was development of methods to isolate anti-malarial agents from rabbit plasma and applying the methods to determine these drugs in 128 samples of rabbit plasma. A second and much more important special project was development of a highly sensitive and specific method to determine squalene in 17 lots of Anthrax Vaccine Adsorbed. As a result of this work, several awards were presented to members of the project team and to SRI International. A third special project was the development and production of a formulated drug product for artesunic acid. Two batches of this drug product were produced and sent to the Army for toxicological evaluations. A poster presentation was made at the 2000 Bioscience Review, and one publication appeared in press.

Respectfully submitted,



Peter Lim
Principal Investigator
Phone: (650) 859-3029
FAX: (650) 859-4321
E-mail: peter.lim@sri.com